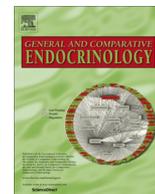


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# General and Comparative Endocrinology

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## Analyzing endocrine system conservation and evolution



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### ABSTRACT

Analyzing variation in rates of evolution can provide important insights into the factors that constrain trait evolution, as well as those that promote diversification. Metazoan endocrine systems exhibit apparent variation in evolutionary rates of their constituent components at multiple levels, yet relatively few studies have quantified these patterns and analyzed them in a phylogenetic context. This may be in part due to historical and current data limitations for many endocrine components and taxonomic groups. However, recent technological advancements such as high-throughput sequencing provide the opportunity to collect large-scale comparative data sets for even non-model species. Such ventures will produce a fertile data landscape for evolutionary analyses of nucleic acid and amino acid based endocrine components. Here I summarize evolutionary rate analyses that can be applied to categorical and continuous endocrine traits, and also those for nucleic acid and protein-based components. I emphasize analyses that could be used to test whether other variables (e.g., ecology, ontogenetic timing of expression, etc.) are related to patterns of rate variation and endocrine component diversification. The application of phylogenetic-based rate analyses to comparative endocrine data will greatly enhance our understanding of the factors that have shaped endocrine system evolution.

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### 1. Introduction

Rates of evolution are tremendously disparate across organisms and their constituent traits. Understanding the basis of this variation can provide valuable insights into the biological constraints that restrict trait evolution, as well as factors that promote trait diversification (Raff, 1996; Kirschner and Gerhart, 1998; Carroll, 2005). Many components of endocrine systems are both multifunctional and essential, thus significant alterations to these systems can result in pervasive pleiotropic effects with deleterious consequences. This is exemplified by the deep evolutionary conservation of many endocrine system components and pathways (Gilbert, 2011; Norris and Carr, 2013). However, endocrine systems are known to be important regulators of growth, differentiation, reproduction, and behavior, which are among the major axes of metazoan evolution (Schluter, 2000; Streelman and Danley, 2003; Coyne and Orr, 2004; Gilbert, 2011; Norris and Carr, 2013). Therefore, variations in endocrine system components are likely to have been major drivers of metazoan diversification. Yet, relatively few comparative studies have quantified the conservation and evolutionary flexibility of endocrine system traits, or tested for the factors that promote their diversification.

Phylogenetic-based comparative analyses are critical for understanding patterns of trait evolution (Felsenstein, 1985; Harvey and Pagel, 1991). The sophistication of these methods and the diversity of questions that they can address have flourished over the last two decades. These include statistical phylogenetic methods to analyze the distributions of traits on a phylogeny, patterns of trait correlations, and rates of trait diversification (Garland, 1992; Martins, 1994; Pagel, 1994; Schluter et al., 1997; Pagel et al., 2004; O'Meara et al., 2006; Beaulieu et al., 2012; Hadjipantelis et al., 2013; Jones and Moriarty, 2013; Adams, 2014; Denton and Adams, 2015; Goolsby, 2015). These analyses have been heavily applied to morphological, physiological, and ecological traits, but rarely to endocrine system traits (some examples discussed below). This may be in part due to the fact that many endocrine system components are inherently dynamic across ontogeny, which challenges quantification. Furthermore, many methods used to quantify endocrine components yield values that are not directly comparable across species. Nucleotide and amino acid based components are, however, readily obtainable and can be directly comparable among even divergent taxa. In parallel with the development of phylogenetic comparative methods, the field of molecular evolution has produced statistical frameworks to analyze patterns and rates of nucleotide and protein substitutions among species (Yang and Bielawski, 2000; Lanfear et al., 2010). The application of high-throughput sequencing methods to the genomes

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and transcriptomes of even non-model species provides the opportunity to collect large-scale comparative data for many evolutionarily relevant endocrine variables (e.g., coding sequences, transcription factor binding locations, cis-regulatory sequences, micro-RNAs, and expression patterns). Consistent quantification of endocrine components across species and analyses of the rates of trait and molecular evolution will significantly enhance our understanding of the factors that drive endocrine system evolution.

This minireview highlights the multitude of points of potential evolutionary variation within endocrine systems, and shows how analyzing the evolutionary rates of endocrine traits, genes, and proteins (individually or across pathways) can elucidate patterns and drivers of endocrine systems conservation and evolution.

## 2. Endocrine system conservation and evolutionary flexibility

Our most intricate knowledge of molecular endocrine mechanisms is primarily based on studies of divergent model organisms, and broad-scale comparisons of these taxa have identified both highly divergent and strictly conserved components of the endocrine system (Heyland et al., 2005). Many features are similar (or identical) in structure or function among highly divergent species, in some cases showing conservation over hundreds of millions of years. For example, thyroid hormone-like molecules and thyroid hormone receptors can be traced to the common ancestor of bilaterians (~700 MYA; Wu et al., 2007; Laudet, 2011; Huang et al., 2015). Functionally, thyroid hormone control of postembryonic remodeling is evident across osteichthians (~430 MYA; Brown, 1997; Norris and Carr, 2013; Shi, 2013), and even short, orthologous genomic response elements for thyroid hormone receptors share gene regulatory mechanisms across divergent tetrapods (~350 MYA; Bagamasbad et al., 2015).

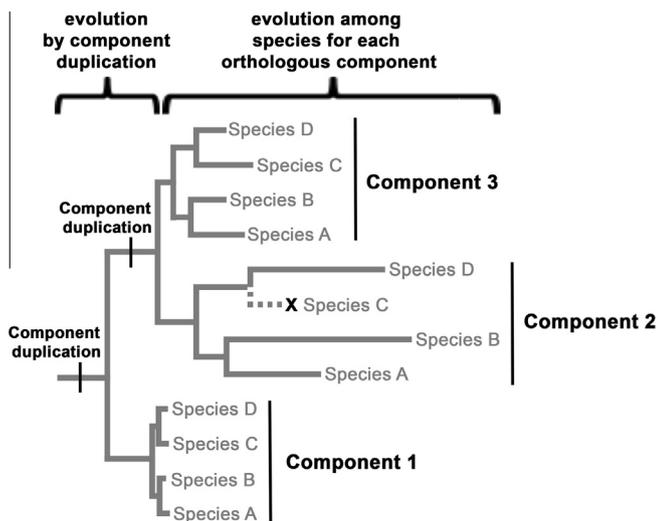
Despite the deep conservation of some endocrine components, this multi-faceted system offers a wealth of material for evolution. Components can diversify along at least two dimensions: (1) through duplication to produce paralogous components, and (2)

through the modification of a given homologous (orthologous) component among species (Fig. 1; Table 1). Even a relatively simple endocrine pathway includes dozens of components that could be changed in composition, direction, magnitude, or ontogenetic timing (Table 2). The composition of components available to an organism is also shaped by the loss (extinction) of ancestral components (Fig. 1), and collectively these evolutionary changes (duplication, modification, and loss) can lead to major phenotypic consequences. Analyzing evolutionary rates of paralogous and orthologous endocrine components (traits) across species in a phylogenetic context allows for a variety of evolutionary inferences, including what factors drive their conservation and diversification.

## 3. Diversification of endocrine traits

Ordinary statistical analyses of trait or genome evolution among species are encumbered by the fundamental problem of phylogenetic non-independence of data points (Felsenstein, 1985). This has been addressed through the development of a variety of phylogenetic comparative methods that can be used to analyze trait or genome evolution while correcting for relatedness of species. Several studies have used phylogenetic comparative methods to test whether variations in endocrine traits are correlated with other phenotypic traits. These include tests for relationships between growth factor levels with life history (Stuart and Page, 2010; Swanson and Dantzer, 2014), reproductive hormones with mating strategies (Garamszegi et al., 2005), and “stress” hormones with ecology (Brischoux et al., 2015), morphology (Lendvai et al., 2013), and social interactions (Abbott et al., 2003). The theory and application of phylogenetic correlation tests to endocrine (signal) data have been recently reviewed (Swanson and Snell-Rood, 2014). Here I review a different, but intimately related, set of phylogenetic comparative analyses that are used to compare rates of trait evolution across clades (Garland, 1992; O’Meara et al., 2006). In the simplest sense, evolutionary rate analyses are based on the premise that trait variation has the potential to change over time. However, biological constraints can restrict trait evolution, whereas the removal (or circumvention) of constraints can provide opportunities for traits to vary (Raff, 1996; Kirschner and Gerhart, 1998; Carroll, 2005). In other words, comparatively low rates of trait evolution (conservation) indicate the action of biological constraints from stabilizing selection, while relatively high rates of trait evolution (flexibility) may indicate relaxed constraints and/or strong directional selection. Analyzing rates of trait evolution across species can be used to test for factors associated with trait conservation and evolution.

The application of phylogenetic-based rate analyses to endocrine traits can address many outstanding questions in endocrine system evolution. For example, how has ecology influenced the diversification of endocrine components? This analysis requires: (1) continuous data that represent variation in an endocrine trait across at least several species (see Table 2 for trait examples), (2) categorical ecological data for the same set of species (aquatic vs. terrestrial in the example; Fig. 2), and (3) a phylogeny for these species with branch lengths that approximate time. Procedurally, the categorical trait (in this case ecology) is stochastically mapped onto the phylogeny in repetition (Nielsen, 2002). These maps are based on the branching structure (topology) of the phylogeny and the distribution of the categorical trait among the included species. In total, the maps represent an estimate of the collective amount of history ( $t$ , time) that the lineages (included in the phylogeny) have exhibited each ecological trait. In other words, the total amount of evolutionary time that one ecological trait was exhibited compared to another across the clade. The rates of change of the continuous trait (in this case the endocrine component) can then be calculated for each ecological category across



**Fig. 1.** Dimensions of component evolution. The two levels of component evolution are by component duplication, and by modification of orthologous components among species. In this example, there were two duplication events in the common ancestor of species A, B, C, and D that resulted in components 1, 2, and 3. Components 1, 2, and 3 have evolved at different rates. Component 1 is the most conserved (shortest branch lengths among species) and component 2 is most variable. Branch lengths represent number of changes. Species C lost component 2 (signified by the X), and therefore only retains components 1 and 3. Functionally equivalent components can also be independently derived in a system through *de novo* evolution (see Section 5).

**Table 1**  
Key terms.

<b>homologous:</b> refers to a trait that is present in different species and has a common origin
<b>orthologous:</b> refers to a gene that is present in different species and has a common origin
<b>duplication:</b> a mechanism of generating multiple copies of a gene within a genome
<b>paralogous:</b> refers to different genes that arose from duplication, which can co-occur in a single species
<b>cladogenesis:</b> process of generating new species or lineages (i.e. new branches on a phylogeny)
<b>diversification:</b> process of increasing diversity by cladogenesis or increasing variance of traits among lineages
<b>synonymous substitutions:</b> mutations to protein-coding genes that do not confer an amino acid change
<b>non-synonymous substitutions:</b> mutations to protein-coding genes that confer an amino acid change
<b>neofunctionalization:</b> the evolution of a new function for a duplicate gene copy
<b>nonfunctionalization:</b> the degeneration of a redundant gene
<b>subfunctionalization:</b> the evolution of more specialized subfunctions by duplicate genes
<b>modularity:</b> process by which components of an organism form semi-autonomous functional units

\* The increase of trait variance among lineages is sometimes referred to as **disparification**, whereas **diversification** is reserved for lineage diversity (e.g., Ackerly, 2009; Burbrink et al., 2012). However, this has only caught on in some disciplines. For simplicity I use **diversification** more generally to refer to both lineages and traits, although I am primarily discussing trait variance here.

**Table 2**

Endocrine components and their potential for evolution. Multi-faceted endocrine systems contain many components, each of which could evolve in several ways. Among species variations in these components (when quantifiable and comparable) represent potential variables that could be analyzed using phylogenetic comparative methods.

Endocrine system component	Potential for evolution – Changes in...
Environmental or endogenous stimuli	...stimulus intensity, stimulus duration, or stimulus type
Endocrine glands	...gland size/shape, ontogenetic/seasonal development of gland
Hormones	...circulating hormone level/persistence, hormone structure
Hormone carriers	...circulating hormone carrier level, hormone binding affinity of carrier
Target cells	...type of target cell, sensitivity of target cell, sensitivity associated with ontogenetic stage or sex
Receptors	...target cell expression, receptor affinity, receptor structure
Response elements	...presence/absence throughout the genome, affinity with receptor DNA binding domains
Coregulators	...activation or repression of transcription factors
Genes regulated	...hormone regulation/dysregulation of genes
Intracellular enzymes	...level of activation/deactivation enzymes
Feedback loops	...positive or negative feedback mechanisms
Interactions	...interactions among system components for additive, synergistic, or antagonistic effects

As discussed in the text, component duplication and loss (Fig. 1, but not listed in this table) can also influence functional evolution, and could be a relevant to most of the components listed above.

the phylogeny under a stochastic model of trait evolution (Fig. 2). Common models used to calculate the rate parameter ( $\sigma^2$ ) include Brownian Motion (BM, a stochastic process) and Ornstein–Uhlenbeck (OU, a stochastic process confined by the strength of selection,  $\alpha$ , around a trait optimum,  $\theta$ ; see Butler and King, 2004 for detailed comparisons). Different rates (and different  $\alpha$ s and  $\theta$ s for OU models) are then calculated for the continuous trait of each ecological group (O'Meara et al., 2006; Beaulieu et al., 2012). Comparatively low rates of evolution for the continuous (endocrine) trait may indicate (ecological) constraints enacting purifying selection, whereas high rates of (endocrine) trait evolution may suggest removal of (ecological) constraints and/or directional selection.

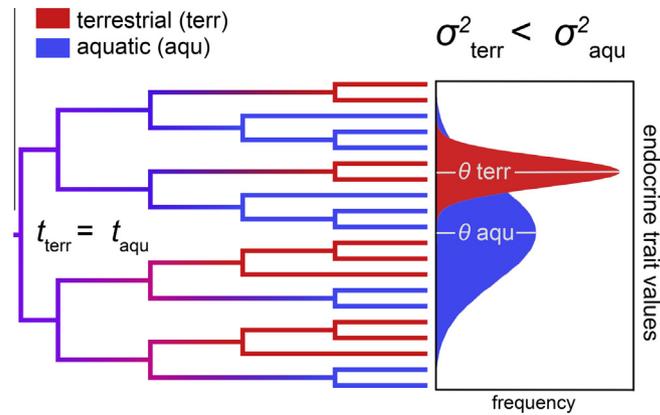
Statistical differences in the rates of continuous trait evolution between lineages that exhibit each of the categorical traits can be determined by comparing the fit of single vs. multi-rate models to the phylogeny (O'Meara et al., 2006; Beaulieu et al., 2012). In this case, the test would determine whether a two-rate model, where the continuous endocrine trait evolves at different rates for lineages that exhibit alternative ecologies, is a better fit than a one-rate model, where the continuous endocrine trait evolves at one rate across the phylogeny. Support for the two-rate model would indicate that alternative ecologies have experienced different rates of endocrine system evolution throughout the history of the clade. From the opposite perspective, this procedure could also be used to test whether endocrine traits have influenced rates of ecological evolution. In other words, have some endocrine traits constrained lineages to certain environments while others allowed for the colonization of broader environments? The interaction between endocrine traits and ecology is just one example, but this

type of analysis could be applied to test whether a wide range of other traits have influenced endocrine component evolution or vice versa. Although beyond the scope of this minireview, it is important to note that patterns of cladogenesis (lineage diversification) and the timing of trait diversification on the tree (e.g., early vs. late) can be used to test how traits evolve with respect to speciation, such as during adaptive radiations (e.g., Harmon et al., 2010; Rabosky and Adams, 2012).

The evolutionary rate analyses described above were developed for univariate, continuous traits where a single value (factor) describes the trait for each taxon. This is not an ideal way to code dynamic, “function-valued traits” that change with respect to other continuous variables such as environmental or endogenous stimuli, ontogeny, body size, etc. (Kirkpatrick and Heckman, 1989; Kingsolver et al., 2015). Recently, methods have been developed to analyze function-valued traits among species using Phylogenetic Gaussian Process Regression (Hadjipantelis et al., 2013; Jones and Moriarty, 2013), and Phylogenetic Comparative Methods (Goolsby, 2015). Both strategies can be implemented to estimate ancestral curves, test for patterns of correlated evolution, and analyze rates of evolution. These are promising developments for analyzing the evolution of function-valued endocrine traits.

#### 4. Diversification of amino and nucleic acid based components

Alignable amino acid and nucleic acid based endocrine components are readily comparable across species through analyses of substitution rates or genetic distances, and these comparisons can even be performed across paralogous components. Protein-based components of endocrine systems are ubiquitous and



**Fig. 2.** Comparisons of evolutionary rates of endocrine system components for species that exhibit alternative ecologies. The phylogeny on the left depicts hypothetical evolutionary relationships of species that exhibit multiple transitions between two alternative ecological modes: terrestrial (red, terr) or aquatic (blue, aqu). The colored branches of the phylogeny represent the probability that the ancestor was aquatic vs. terrestrial based on a summation of stochastic character maps for the ecological trait. In this simplified example, there are equal numbers of aquatic and terrestrial species distributed across equally proportional branches, so the total amount of time ( $t$ ) that the clade exhibited each ecology is equal ( $t_{\text{terr}} = t_{\text{aqu}}$ ). The frequency distributions for a continuous endocrine trait to the right show differences in trait optima ( $\theta$ ; means) for each ecological mode, and also substantially greater variance in for aquatic compared to terrestrial species. Rates of trait evolution are a calculation of variance over evolutionary time. Given the equal amounts of aquatic and terrestrial history of this clade, the example also shows that aquatic lineages had higher rates of evolution for this endocrine trait ( $\sigma_{\text{terr}}^2 < \sigma_{\text{aqu}}^2$ ; see text for a description of how analyses are implemented and statistically compared).

include: some hormones, systemic hormone carriers, hormone receptors, transcriptional co-factors, chaperones, and enzymes (Table 2; Gilbert, 2011; Norris and Carr, 2013). At the nucleotide level, non-synonymous substitutions are changes that confer an amino acid substitution, while synonymous (silent) substitutions are considered neutral, and therefore approximate the background mutation rate. The non-synonymous ( $D_N$  or  $K_A$ ) to synonymous ( $D_S$  or  $K_S$ ) nucleotide substitution ratio ( $\omega$ ) reflects the rate at which mutations between species result in amino acid substitutions (Miyata and Yasunaga, 1980; Li et al., 1985; Nei and Gojobori, 1986; Yang and Bielawski, 2000). This ratio can also be used to assess the direction and degree of selection compared to neutral evolution ( $\omega = 1$ ), where  $\omega > 1$  indicates positive selection and  $\omega$  between 0 and 1 suggests that the gene has been biased by negative (purifying) selection (i.e. constraint; Kimura, 1977; Yang and Bielawski, 2000).

Significant shifts in amino acid substitution rates on different branches of the phylogeny can be evaluated by comparing the fit of a model that implements a single  $\omega$  value across the tree to models with  $\omega$  as a free parameter among lineages, or to models that allow different  $\omega$ s among groups of lineages (Yang and Nielsen, 1998; Yang and Bielawski, 2000). Associations between substitution rate patterns and categorical or continuous variables can be tested with phylogenetic correction through paired species comparisons or whole tree comparisons (Lanfear et al., 2010). Furthermore, since the individual amino acids that comprise a protein sequence are potentially independent, procedures have also been developed to allow for substitution rate heterogeneity among codon positions and among branches of the phylogeny (Yang and Nielsen, 2002; Zhang et al., 2005). This approach and also some Bayesian adaptations have been developed to identify positions that exhibit strong patterns of selection (Yang et al., 2005; Murrell et al., 2013; Huang and Golding, 2015). For example, Sparkman et al. (2012) showed that the rate of evolution of Insulin-like growth-factor-1 in Lepidosaurs (clade that includes lizards and snakes) was substantially higher than in other reptiles. They also found that many sites in the domain for receptor binding (C-domain) showed evidence of positive selection.

Many non-coding nucleotide sequences also play pivotal roles in endocrine systems. These include enhancers, promoters, hormone receptor response elements, untranslated regions of transcripts, and microRNAs (Table 2). Genetic distance estimates

under a wide range of possible nucleotide substitution models are often used to describe variation among nucleotide-based sequences (Page and Holmes, 1998). The relative conservation of some non-coding regions has in some cases provided the first line of evidence for their functionality (e.g., ultra-conserved elements, Bejerano et al., 2004). The calculation of nucleotide substitution rates requires an understanding of the divergence times among species in the comparison (Tajima, 1993). With more recently developed procedures, divergence times can be incorporated into substitution rate analyses by implementing time calibration points (e.g., ages based on fossils) on at least some nodes in the phylogeny. Substitution rates can then be estimated among branches of the tree through Penalized Likelihood (Sanderson, 2002) or Bayesian coalescent methods (Drummond and Suchard, 2010). Similar to approaches using protein-coding sequences, the factors that affect nucleotide substitution rates can be tested using phylogenetic comparative methods. Additional methods that have been developed to test for differences in evolutionary rates of short non-coding sequences are discussed below.

## 5. Diversification by duplication and *de novo* evolution of endocrine components

Previous sections cover the diversification rates of orthologous components across species. However, patterns of component diversification can accumulate along an additional dimension of complexity, via gene/genome duplication (Fig. 1). In addition, functionally equivalent components can be repeated in a system via *de novo* evolution. Redundant components have several possible fates (Force et al., 1999; Lynch and Conery, 2000): (1) the retention of both copies, which can increase dosage or responsiveness, (2) the evolution of a new function for one of the otherwise redundant copies (neofunctionalization), (3) the degeneration of one of the redundant copies (nonfunctionalization), or (4) shifts in the subfunctions of both copies (subfunctionalization), which collectively allows for the original function to be maintained but with a greater degree of specialization by each component.

Phylogenetic analyses have shown that gene and whole genome duplications have been paramount in the evolution of the metazoan endocrine system, especially for the diversification of ligands and receptors such as Opioids and G-protein coupled receptors

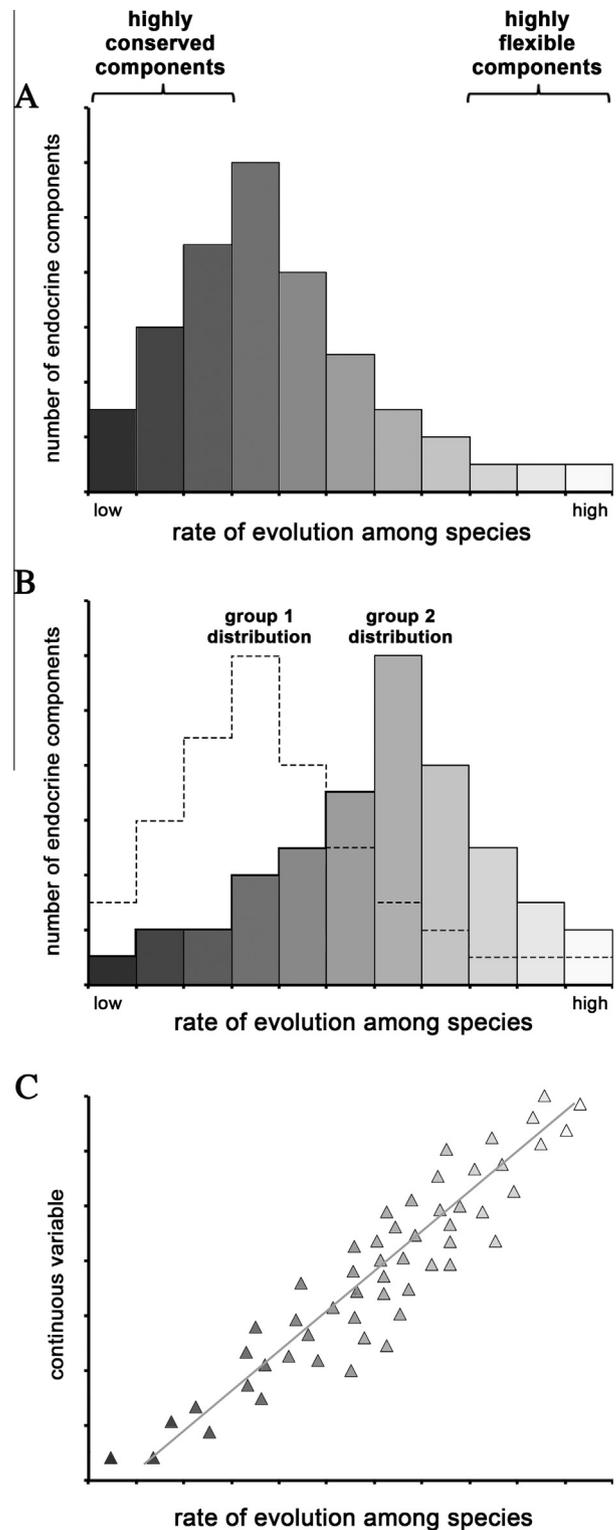
(Sundström et al., 2010; Katritch et al., 2012; Dores et al., 2014), Cytokines and Cytokine Receptors (Ouyang and He, 2003; Denver et al., 2011), and the superfamily of Nuclear Receptors (Bertrand et al., 2004; Thornton, 2001). Phylogenetic-based rate analyses have important applications for interpreting the evolution of duplicated genes. For example, the demonstration of heterogeneous substitution rates among paralogous genes provides evidence of functional divergence among the duplicates (Gu, 1999, 2001; Huang and Golding, 2012). Furthermore, ancestral state reconstructions, which are in part predicated on rates of character change across the tree, can be used to determine the structure and interactions of historical components (Thornton, 2004; Harms and Thornton, 2010; Groussin et al., 2013). Based on these reconstructions, extinct components can be resurrected in the lab to test their functionality, and provide an understanding of the key features that guided the evolution of descendant copies (Ortlund et al., 2007; Harms and Thornton, 2014).

In addition to origination by duplication, short non-coding DNA components with high functional degeneracy, such as nuclear receptor response elements, also have a high propensity for *de novo* evolution throughout the genome. High-throughput sequencing of genomic fragments enriched by chromatin immunoprecipitation (ChIP-Seq) has been a critical advance for global mapping of transcription factor bound or transcriptionally active regions. Thus far, relatively few studies have compared ChIP-Seq data across species (e.g. Stefflova et al., 2013; Villar et al., 2015), but the findings have been important for understanding the evolution of repeated regulatory elements. In a recent study, Villar et al. (2015) performed ChIP-Seq analyses of acetylation and methylation proteins in the liver cells of 20 mammal species, and collectively identified >35,000 putative regulatory regions. They quantified the degree of conservation of each region based on the number of species that have the region present (ChIP enriched and alignable), and found that enhancers have much higher turnover rates compared to promoters. Very few of the enhancers contained signatures of transposable elements, which in part lead them to conclude that enhancers primarily arose via *de novo* evolution as opposed to duplication during transposable element proliferation.

Even though the half-lives of many short promoters are fast, there are still many that are persistent across clades, and their evolutionary rates can be analyzed by nucleotide substitution methods described above, or by comparisons to outgroup species (Wagner et al., 2004). ChIP-Seq analyses of nuclear hormone receptors across species (in conjunction with RNA-Seq experiments) will provide powerful data sets for testing patterns of response element evolution. These analyses will ultimately serve as the principle link between hormones and the evolution of transcriptional regulation.

## 6. System-wide diversification rates

It is apparent that some types of endocrine components are strictly conserved across species while other components are highly variable, but quantitative comparisons of these differences in evolutionary rate heterogeneity have been limited to relatively few examples. Comparative evolutionary rate estimates can be used to identify constrained and evolutionary flexible components, and also can provide a critical framework to test the drivers of endocrine component evolution across systems (e.g., Lachowicz et al., 2015; McGaugh et al., 2015). One way to approach this problem is to first estimate the rates of evolution for individual endocrine components across species (as described above), and then compare the rate values of collections of different components sampled from across pathways or the entire system. This provides quantifiable values for the degree and frequency of rate differences among endocrine components (Fig. 3A). Furthermore the means and



**Fig. 3.** Comparative rates of evolution of different endocrine system components among groups and their correlations with other continuous traits. Rates of evolution across species are calculated for each of many comparable endocrine components (e.g. protein coding genes). The distribution of rates across components is plotted as a histogram (Fig. 3A and B), to show their relative rates of evolution. The left side of the distribution shows highly conserved components (dark bars) and the right side shows highly flexible components (light bars). Fig. 3B shows differences in the distributions of rates of component evolution compared among groups (e.g. clades, ecologies, or other categorical groupings). The dashed line shows the rate distribution of Fig. 3A to compare it to the distribution plotted on 3B. Rate variation estimates for different components can also be analyzed with respect to other continuous traits (Fig. 3C), to test for system-wide parameters that may influence component diversification (see text for examples).

variances of the rate distributions can be compared among different categorical groups. This could include comparisons of the same set of components for taxa sampled from different clades, or for groups of unrelated taxa that share categorical attributes (e.g., the same ecology). Similar analyses could also be used to test how categorical attributes of the components themselves influence their rates of evolution (Fig. 3B, and example below).

In a recent exemplar study, [McGaugh et al. \(2015\)](#) compared rates of evolution of 61 protein-coding genes from the insulin/insulin-like signaling and target of the rapamycin network across 66 amniotes. Among other insights, their study showed that genes coding for extracellular components, such as hormones, receptors, and binding proteins, evolved at faster rates than intracellular components in this pathway. Using a similar strategy, [Lachowicz et al. \(2015\)](#) showed that the protein sequences of mammalian kinases that strongly interact with Heat Shock Protein 90 (clients) have higher rates of evolution than weak-client or non-client kinases. Many of the approximately 200 kinases examined in this study are involved in endocrine pathways ([Lachowicz et al., 2015](#)). These studies are examples of how characteristics or interactions of endocrine system proteins influence their rates of evolution. Such analyses could be extended to many other pathways and problems. For example, a fundamental problem of evolution and development is how the processes of modularity and integration influence rates of trait evolution ([Wagner et al., 2007](#); [Zelditch et al., 2009](#); [Goswami et al., 2014](#); [Denton and Adams, 2015](#)). The interacting components of endocrine signaling pathways can be realized as modules with varying degrees of autonomy among pathways ([Heyland et al., 2005](#)). System-wide analyses can be used to test how the formation or autonomy of endocrine modules affects the evolutionary rates (and directions) of constituent components.

The effects of continuous variables on rates of endocrine component evolution can also be analyzed across systems to test how endocrine system expression and architecture influences patterns of component conservation and evolution (Fig. 3C). For example, greater pleiotropic effects are expected from aberrations to genes (or other components) that are expressed in early development ([Raff, 1996](#); [Good and Nachman, 2005](#)) or in a greater diversity of tissues ([Duret and Mouchiroud, 2000](#)). Therefore, these components should exhibit lower rates of evolution across species than those expressed later in development or in a more limited set of tissues. Another potentially important relationship based on proteins, albeit controversial, is whether the number of interacting components influences rates of evolution ([Fraser et al., 2002, 2003](#); [Teichmann, 2002](#); [Jordan et al., 2003](#)). From an endocrine perspective, if the number of component interactions predicts component evolution, it would be expected that components with more interactions among pathways would have lower rates of evolution compared to those with fewer interactions. These are just a sampling of the testable predictions that can be analyzed by comparing the rates of evolution of different endocrine components across species, then testing for correlations between the rates and factors that may govern their variation.

## 7. Summary

Relatively few studies have explicitly tested rates of endocrine component evolution in a phylogenetic context, even though such analyses are key to understanding patterns of diversification of the endocrine system. This may be in part due to historical and current data limitations for most groups, however recent high throughput technologies (e.g., RNA-Seq, ChIP-Seq, genome sequencing) provide the potential to rapidly collect comparable data sets across species for a variety of evolutionarily relevant endocrine variables. Furthermore, development of methods for coding and analyzing

function-valued endocrine traits will facilitate evolutionary analyses of functionally dynamic endocrine components. This is a tremendously fertile area for future research and will significantly enhance our interpretations of the evolutionary processes that dictate patterns of endocrine system diversification.

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